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Bio-based production of crotonic acid by pyrolysis of poly(3-hydroxybutyrate) inclusions

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ABSTRACT

Bio-based material development has become a new focus globally due to limited supply, increasing price of fossil fuel, and demands for environment sustainability. Current industrial production of crotonic acid through petrochemical route has several drawbacks: i) non-renewable, as it is derived from petroleum resource, ii) involves numerous complicated steps, and iii) produces low yield. Therefore, this paper proposes a method for production of bio-based crotonic acid by direct pyrolysis of bacterial poly(3-hydroxybutyrate) inclusion as an alternative to the petrochemical route. Thermogravimetric profile of poly(3-hydroxybutyrate) inclusions showed poly(3-hydroxybutyrate) degradation occurred at a temperature range of 270 °C–350 °C with maximum degradation rate at 310 °C. Analysis of products from isothermal pyrolysis of poly(3-hydroxybutyrate) at 310 °C revealed that pyrolysis of poly(3-hydroxybutyrate) inclusions yielded approximately 63% of crotonic acid. This is 30% higher than the conventional crotonic acid production via petrochemical method. The proposed method also offers other benefits such as renewable and simpler in processing. Besides, by-products of fermentation and pyrolysis are easy to treat, thus minimizing threat to the environment. Moreover, demands for bio-based products are expected to rise in the near future because of social, environmental and economical issues related to fossil resources which make bio-based production method more appealing and favourable. Therefore, pyrolysis of bacterial poly(3-hydroxybutyrate) inclusions provides new insight of renewable and green chemistry of the crotonic acid production.

1. Introduction

The attention of researchers has now been shifted towards the discovery of alternative pathways and products which are renewable and environmental friendly due to depletion of natural hydrocarbon depository and the environmental pollution caused by its utilization (Jong et al., 2012). One of the examples of bio-based product is poly(3-hydroxybutyrate) (PHB). PHB is a polyester which belongs to the polyhydroxyalkanoates (PHA) group (Gonzalez et al., 2005). The appealing properties of PHB include biodegradability, derived from biological and renewable resources and have similar properties to petroleum-derived polypropylene, thus making it a suitable substitute for non-degradable petroleum-derived plastics (Tokiwa and Calabia, 2004). In the last few years, our group has published several papers regarding PHA for sustainable environment especially on the production (Hassan et al., 2013; Zahari et al., 2014, 2012b) and its recovery (Mohammadi et al., 2012a, 2012b; Zahari et al., 2012b). Despite of its biodegradability, PHB homopolymer is thermally instable causing it to have narrow processing window (Ariffin et al., 2008). A lot of research have been conducted focusing on thermal degradation of PHB in order to examine and clarify its thermal degradation mechanisms (Tokiwa and Calabia, 2004; Gonzalez et al., 2005; Ariffin et al., 2008).

Thermal degradation of PHB has been known to proceed mostly under β-elimination mechanism and produces dehydrated

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monomer, crotonic acid as a major product (Ariffin et al., 2008). We previously reported that selective formation of crotonic acid can be obtained when $Mg(OH)_2$ is used as catalyst during thermal degradation of PHB (Ariffin et al., 2010). Crotonic acid has several specific purposes especially in the synthesis of copolymers. The most significant derivatives of crotonic acid are crotonic acid-vinyl acetate copolymers which have commercial names include Cevian, Gelva, Mowilith and Vinac (Schulz et al., 2003). Crotonic acid-vinyl acetate copolymers are commonly used in cosmetic and hair styling products as to impart sheen to the applied features as well as to give desired hair shape (Campain, 2010). Besides, crotonic acid derivatives also have applications in other industries such as coating, paint, textile, binders, adhesives, flocculants, ceramics and agrochemical industries (Schulz et al., 2003; Jasicka-Misiak et al., 2005). Crotonic acid and its esters also have antimicrobial characteristics and thus could be employed in deodorants (Van Walsem et al., 2011). Above all, geometric-selectivity of the trans-crotonic acid and its esters are believed to become crucial monomers for production of optically-active polymers in the near future (Ute et al., 2003).

Despite numerous applications of crotonic acid in various fields, its current production via petrochemical pathway has somehow become its drawback as the resource is non-renewable. Commercial crotonic acid is derived from oxidation of crotonaldehyde, which is the byproduct from petrochemical conversion of hydrocarbon into ethylene (Arpe, 2010; Rittner et al., 1990; Schulz et al., 2003). Meanwhile on a non-industrial scale, crotonic acid can be prepared by several methods such as dehydration of 2-hydroxybutanoic acid, photochemical oxidation or oxidative irradiation of crotonaldehyde by ultrasound, isomerization of vinylacetic acid with sulfuric acid and oxycarbonylation of propene with transition metal complex catalysts (Schulz et al., 2003). Nevertheless, all these methods involve the use of non-renewable resources. Due to the increasing awareness on the importance of sustainable and renewable bioproducts, in addition to the raising price of fossil resources, the need for bio-based crotonic acid has caught attention of many researchers. Recently, a couple of patents has been filed related to biological-based crotonic acid production (Koch and Meurer, 2012; Mauch and Schmid, 2008). Mauch and Schmid (2008) developed transgenic cell preferably elected from bacterial species of *Ralstonia eutropha, Escherichia coli, Corynebacterium glutamicum* and *Clostridium acetobutilicum*. The invention manipulates bacterial 2-oxoglutarate pathway with over expression of specific enzymes such as 2-hydroxyglutarate dehydrogenase, glutaconate-CoA transferase, hydroxyglutaryl-CoA dehydratase and glutaconyl-CoA decarboxylase to produce crotonic acid. Meanwhile, Koch and Meurer (2012) developed a recombinant cell with elevated activity of enzymes involved in 2-ketoglutarate pathway (*i.e*: 4-hydroxybutyryl-CoA dehydratase and crotonate-CoA transferase). Both of the inventions enable production of crotonic acid through bacterial fermentation. However, there was no information on the quantitative yield and purity of crotonic acid produced from those methods. Furthermore, a proper isolation method will be needed in order to recover crotonic acid from fermentation broth.

The present study thus aimed at producing crotonic acid through a green and simpler procedure by exploiting thermal degradation pathway of PHB. In this study, direct pyrolysis of PHB inclusion obtained from fermentation of *Cupriavidus necator* NCIMB 11599 was carried out in order to produce crotonic acid. The yield of crotonic acid obtained was calculated based on PHB content produced in *C. necator* NCIMB 11599. Finally, a comparison between the proposed bio-based and conventional petroleum-based methods in terms of the availability of raw materials, number of steps involved, crotonic acid yield, and economic evaluation in term of production cost estimation as well as market potential for bio-based crotonic acid was also discussed in this article in order to clarify the feasibility and sustainability of the proposed method for bio-based crotonic acid production.

2. Materials and methods

PHB was bought from Sigma–Aldrich (Germany) and used as standard sample. All other chemicals were of the highest purity commercially available and were used without further purification unless otherwise stated.

2.1. Cultivation of C. necator NCIMB 11599

C. necator NCIMB 11599 was grown in Mineral Salt Media (MSM) in a 20 L bioreactor Biostat Cplus (Sartorius, Germany) at 30 °C (Zahari, 2013a). Fermentation was performed using glucose as carbon source under phosphate limitation condition in order to stimulate PHB granule accumulation inside the cell. Cells containing PHB were then harvested by centrifugation in a Thermo Scientific Sorvall Legend XTR Centrifuge. The cells were then frozen overnight before lyophilized using VirTisBenchTop K freeze dryer. PHB content was determined by GC (Zahari et al., 2012a). GC analysis showed that the cells contained approximately 66 ± 3% PHB.

2.2. Determination of PHB inclusion degradation temperature by thermogravimetric analysis (TGA)

Approximately 6–9 mg of PHB sample was put on the aluminum pan and set in a thermogravimetric analysis (TGA) (model TGA − 7 from Perkin Elmer, USA). The sample was heated at heating rate of 10 °C/min in the range of 30–550 °C under steady flow of nitrogen (20 ml/min). A blank aluminum pan was used as reference. Each experiment was conducted 3 times.

2.3. Isothermal pyrolysis in a glass tube oven

Apparatus arrangement for isothermal pyrolysis is shown in Fig. 1. About 500 mg of sample was subjected to isothermal pyrolysis in a Sibata GTO-2000 glass tube oven. The oven was heated in two steps; first, the oven temperature was increased from room temperature to 200 °C and then kept at these temperatures for 30 min in *vacuo* in order to ensure air was completely removed and to reduce the impurities from degradation of bacterial cell components in the product collector. For the second step, the temperature was further increased to samples' maximum degradation temperatures, *i.e.* 290 °C and 310 °C for PHB and PHB inclusions respectively, and the temperature was again kept constant for 30 min (Ariffin et al., 2010). Vaporized pyrolyzates were condensed and the obtained products were analyzed by GC–MS and ^{1}H NMR for characterization. Recovery yield of crotonic acid production was calculated based on below's equation:

$$\text{Recovery yield} = \frac{\text{Amount of crotonic acid collected(g)}}{\text{Theoretical amount of crotonic acid(g)}} \quad (1)$$

where theoretical amount of crotonic acid produced from thermal degradation was a complete conversion of PHB i.e. 1 g of PHB will be produce 1 g of crotonic acid.

2.4. Gas chromatography mass spectrometry (GC–MS)

Pyrolyzates from isothermal pyrolysis were analyzed using Perkin Elmer Clarus 600 gas chromatography-mass spectrometer. High purity helium gas was used as carrier gas at a constant flow

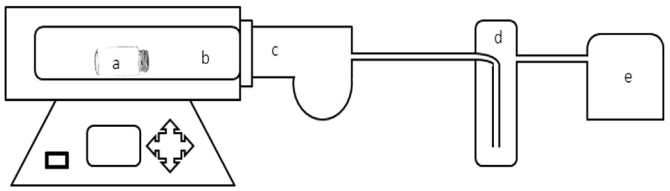

Fig. 1. Pyrolysis apparatus for crotonic acid production; (a) sample vial, (b) heating chamber, (c) collector, (d) cold trap, (e) vacuum pump.

rate of 6 ml/min. Pyrolyzates were dissolved in chloroform prior to analysis and were introduced into MS through 5% Phenyl Polysilphenylene-siloxane column; 30 m × 0.25 mm I.D × 0.25 μm film thickness (BPX-5, SGE analytical science). The ion source temperature used for MS was 200 °C. The data was taken from 3 min until 32 min. Each experiment was conducted in triplicates.

2.5. Proton-NMR (^{1}H NMR) spectroscopy

Chemical composition of pyrolyzates was also determined by ^{1}H NMR. The spectrum was recorded on a JEOL NMR 500 MHz system. Chloroform–*d* ($CDCl_3$) was used as solvent. Chemical shifts were reported as δ values (ppm) relative to internal tetramethylsilane (TMS) in $CDCl_3$ unless otherwise noted. The expected ^{1}H NMR chemical shifts were predicted using a ChemNMRprogram in a CS ChemDraw Ultra version 6.0 (Ariffin et al., 2008).

2.6. Crotonic acid market price

Crotonic acid is the main product of PHB pyrolysis. It forms white to yellow crystals during deposition of volatile crotonic acid. Because of the crystal nature of crotonic acid, it can be separated with ease and thus the costs involves during recovery are negligible (Kuppens et al., 2010).

There is no exact information available about current production cost of crotonic acid. Therefore in this study, the production cost of crotonic acid will be assumed to be the similar to sales price of large quantities of crotonic acid offered by the suppliers. According to Kuppens et al. (2010), the price of crotonic acid is approximately 5–10 EUR kg^{-1} or 6.75–13.5 USD (currency rate of Euro/USD is ≈ 1.35 in 2014).

2.7. PHB biomass production cost

In this study, estimation of PHB biomass production cost was based on a model reported by Zahari (2013b) which includes capital and operating cost. The method was selected due to the similarity in bacteria strain used and method of PHB production. PHB recovery cost was however included in the calculation, which is not relevant to the current study. Therefore, the cost estimation may exceed the actual production cost. Based on Zahari (2013b), the production cost of PHB produced from oil palm frond (OPF) juice was approximately USD 3.31/kg PHB.

2.8. Pyrolysis cost

Pyrolysis cost estimation was calculated based on equation (2) as proposed by Bridgwater et al. (2002). The equation takes into account the cost for development of a pyrolysis reactor, feeding system and product recovery.

$$\text{Pyrolysis cost} = 4.0804 \times 10^4 \times \left(\Omega \times 10^3\right)^{0.6194} \quad (2)$$

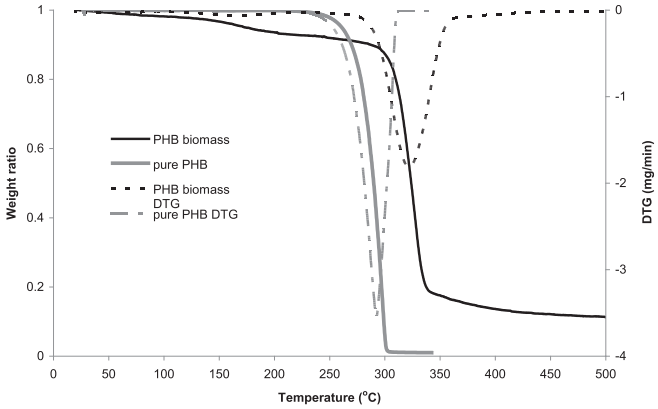

Fig. 2. Degradation profile of PHB and PHB inclusion.

where Ω is the mass flow rate of PHB biomass in oven dry tonne/hour (odt/h).

For the purpose of this study, Ω was estimated based on 10,000 tonnes of PHB biomass pyrolyzed annually with 16 h working time for six days per week. The value of Ω is 2.0 odt/h.

3. Results and discussion

In this study, the results were discussed in three different sections. The first section discussed on thermal degradation behavior of all samples. This is followed by discussion on pyrolysis products and recovery yield of crotonic acid. Finally, biobased method was compared to petrochemical method to examine the sustainability of both methods.

3.1. Thermal degradation temperature of PHB samples

Thermal degradation behaviors of PHB and PHB inclusions were examined using TGA by measuring the weight loss of the samples over reaction temperature. According to data shown in Fig. 2, TG curves of all samples demonstrated a smooth decomposition from beginning until the end of the process. PHB sample was fully degraded and no carbonaceous residue was left. It can be observed from the curve that PHB showed one-step degradation whereas PHB inclusions showed at least two-step degradation.

Table 1
Composition of PHB pyrolyzates.

	PHB	PHB inclusion
Pyrolysis temperature (°C)	290	310
Pyrolyzates recovery wt. = %	100	82
Crotonic acid yield wt. = %	62.5	63.7
Product composition		
GC–MS analysis (%)		
Component		
trans-crotonic acid	63.4	50.5
cis-crotonic acid	1.3	3.5
3-HB	1.6	2.8
Dimer	28.6	30.6
Trimer	5.1	9.4
Others	–	3.2
^{1}H–NMR analysis (%)		
Component		
trans-crotonic acid	63.8	51.7
cis-crotonic acid	1.0	2.8
3-HB	1.5	2.7
Oligomers (dimer & trimer)	33.7	39.6
Others	–	3.2

Table 2
Bacterial strain and carbon sources for PHB production.

Strain	Carbon sources	PHB content (wt%)	Reference
Bacillus megatarium	Beet molasses, date syrup	~50	Omar et al. (2001)
Alcaligenes latus DSM 1124	Soya waste, malt waste	33,71	Yu et al. (1999)
Bukholderia sp. USM(JCM 15050)	Palm oil derivatives, fatty acids, glycerol	22–70	Chee et al. (2010b)
Cupriavidus necator	Bagasse hydrolysates	54	Yu and Stahl (2008)
Cupriavidus necator DSM 545	Waste glycerol	50	Cavalheiro et al. (2009)
Cupravidus necator NCIMB 11599	Saccharified waste potato starch	46	Haas et al. (2008)
Cupriavidus necator CCUG 52238^T	Oil palm frond juice	32	Zahari et al. (2012b)
Cupriavidus necator CCUG 52238^T	Oil palm frond juice	44	Zahari et al. (2012a)
Cupriavidus necator CCUG 52238^T	Oil palm frond juice	42	Zahari (2013a)

This is expected because PHB inclusions consist of numerous constituents apart from PHB such as cell components of bacteria. It is also interesting to note that black residue was left after pyrolyzation of PHB inclusions which might represent non-volatile carbonaceous materials of bacterial cells at employed temperature range. Weight loss at temperature below 110 °C corresponded to the loss of water from the biomass, while at 110–270 °C was due to decomposition of bacterial cell components which contributed to the presence of impurities in the pyrolyzate (Table 1). PHB degradation temperature ranges for PHB and PHB inclusion were recorded at 230–310 °C and 270–350 °C, respectively, showing that PHB in biomass was more stable than pure PHB. Similar result was reported by Kopinke et al. (1996). The differences in thermal stability between PHB and PHB inclusions could be due to limitation in thermal diffusion in PHB inclusions, as well as different PHB characteristics, *i.e.* molecular weight. Based on the degradation profile, temperatures of ~290° and ~310 °C (maximum degradation temperature) were selected as reaction temperatures for isothermal pyrolysis of PHB and PHB inclusion in glass tube oven.

3.2. Crotonic acid recovery

Isothermal pyrolysis was conducted in a glass tube oven and the pyrolysis products were analyzed using GC–MS and ^{1}H NMR. Total ion current (TIC) chromatograms of the analyzed products are shown in Fig. 3. Similar pattern was observed for pyrolysis products from both PHB and PHB inclusion samples with three major peaks at retention time of 4–8 (peak 1), 12–14 (peak 2) and 18–19 min (peak 3) corresponded to dehydrated monomer, dimer and trimer of PHB (Morikawa and Marchessault, 1981; Watt et al., 1991; Kopinke et al., 1996; Kopinke and Mackenzie, 1997; Aoyagi et al., 2002; Ariffin et al., 2010). Characteristic signals at m/z = 41, 68, 86 for peak 1, m/z = 41, 69, 87, 103,154 for peak 2 and m/z = 43, 69, 87,103, 155, 171, 259 for peak 3 confirmed the result. Minor peaks near peak 1 were observed and identified as iso-crotonic acid and 3-hydroxybutyric acid based on their characteristic signals. Even though iso-crotonic acid has similar molecular formula with crotonic acid, difference in geometric orientation causes different boiling points, and therefore different retention time (Kopinke et al., 1996). Minor peaks were also observed for dehydrated

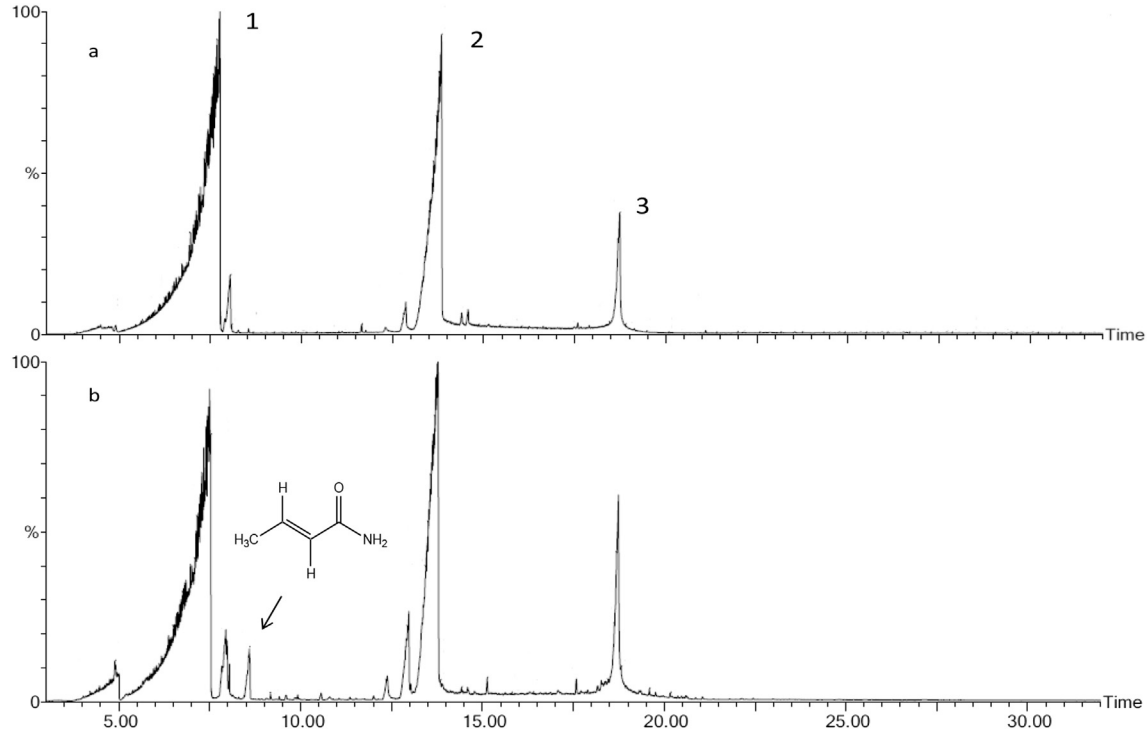

Fig. 3. Total ion current (TIC) chromatogram of pyrolyzates of (a) pure PHB (pyrolysis at 290 °C), (b) PHB inclusion (pyrolysis at 310 °C). Arrow shows new peak observed which has been identified as crotonamide.

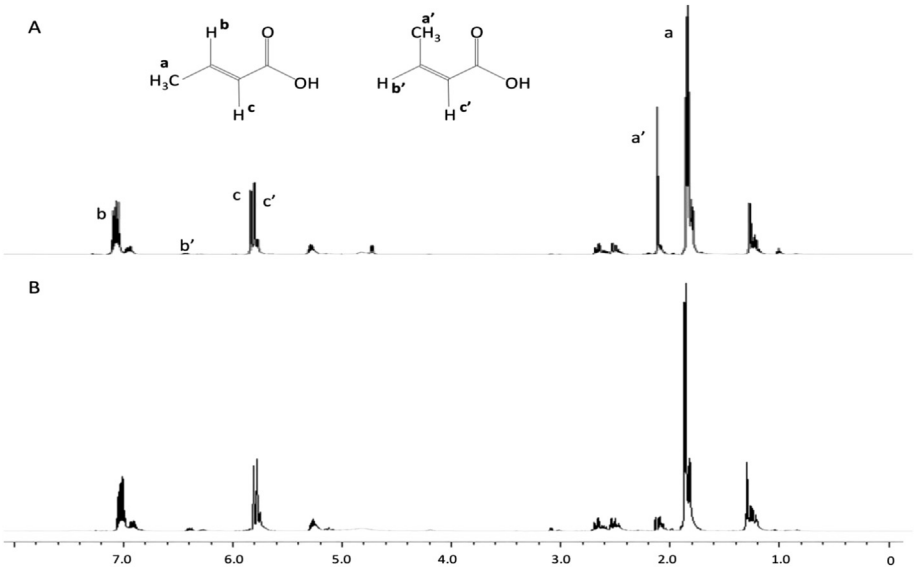

Fig. 4. ^{1}H NMR spectra of pyrolyzates of (a) pure PHB, (b) PHB inclusion.

dimer and trimer which were identified as their respective isomers (Kopinke et al., 1996; Kopinke and Mackenzie, 1997). No higher oligomers were detected. Oligomers higher than trimer might not be detected due to secondary reactions or the oligomers are not volatile at the temperature used during the analysis (Gonzalez et al., 2005). It is interesting to note that pyrolyzates of PHB inclusion showed an additional peak at 8.5 min which was assigned to crotonamide. Based from its chemical structure, the crotonamide is expected to be produced from secondary reaction of crotonic acid and other cell components.

The pyrolyzates were also analyzed for their composition and chemical structure using ^{1}H NMR (Fig. 4). Appointments of the ^{1}H NMR peaks were done according to the previous reports (Ariffin et al., 2008; Morikawa and Marchessault, 1981). Results obtained from ^{1}H NMR analysis were comparable to those from GC–MS, confirming the composition of the pyrolyzates. Overall material balance for the pyrolysis of PHB and PHB inclusion is shown in Fig. 5.

Quantitative information of the pyrolysis is summarized in Table 1. As shown in Table 1, pyrolyzates collected from pyrolysis of PHB and PHB inclusions were 100% and 82%, respectively. These results are in agreement with TG curve (Fig. 2) which showed residual weight of about 18% after thermal degradation of PHB inclusions. Meanwhile, it is interesting to note that even though both of the PHB and PHB inclusion samples were pyrolyzed at their corresponding maximum degradation temperature, the composition of crotonic acid (*cis* and *trans*) in their respective pyrolyzate was quite different with only ~53% crotonic acid for PHB inclusions compared to ~65% for PHB sample. This was contributed by secondary reaction of crotonic acid with other degradation product from cell component to form crotonamide (Fig. 3b). Besides, the composition of *cis*-crotonic acid for PHB inclusion (3.5%) was higher than PHB (1.3%), which may be due to higher pyrolysis temperature used, which could increase the possibility for side-reactions to occur to form *cis*-crotonic acid (Ariffin e al., 2010). Nevertheless, crotonic acid yield for both pure PHB and PHB biomass were almost similar (~63%). It is believed that conversion of PHB into crotonic acid can be increased considerably if the thermal degradation proceeded with the aid of catalysts such as magnesium oxide and magnesium hydroxide as reported by Ariffin et al. (2010).

3.3. Comparison between bio-based and petrochemical-based crotonic acid

As for this study, several aspects regarding proposed bio-based crotonic acid and conventional petrochemical-based method are compared in order to evaluate sustainability characteristics between the two methods. Availability of starting materials, process steps, crotonic acid yield and production cost were focused in this study.

3.3.1. Availability of raw materials for the production of crotonic acid

Table 3 shows process steps for petrochemical-based and bio-based crotonic acid. As seen in Table 3, crotonic acid is currently derived from non-renewable petroleum resource through cracking process. The hydrocarbon produced from cracking process, *i.e.* ethylene was then oxidized to produce acetaldehyde, before being condensed for the production of crotonaldehyde and finally crotonic acid. Biological production of crotonic acid as proposed in this study which involves PHB fermentation from renewable resource, for example oil palm frond juice (Zahari et al., 2012b) followed by direct pyrolysis of the PHB inclusions. The differences between the two pathways lie not only on the number of steps and methods involved, but also the type of feedstock used. Current petrochemical-based crotonic acid involves the use of nonrenewable feedstock which is the petroleum. Increasing demands for energy source and raw materials for chemical product have increased the consumption of petroleum worldwide. Fig. 6 shows that the world's petroleum consumption showed a steady increment from 76784.1 $\times$ 10^3 barrels per day in 2000–89406.6 $\times$ 10^3 barrels per day in 2012 (EIA, 2013). Even though many new oil wells are discovered which consequently increase the oil production and reserves, it has been estimated that with current consumption rate, the crude oil reserve will only last for 42 years (Arpe, 2010).

Despite the fact that numerous efforts and researches have been done to lengthen the supply of the petroleum, alternative methods need to be discovered to sustain the chemical industry. Therefore, current research is now shifted on bio-derivative products which rely on the renewable resources. The proposed method to produce crotonic acid via pyrolysis matches current research focus because carbon source for bacterial fermentation of PHB can be obtained from a variety of renewable resources such as plant oils, fatty acids

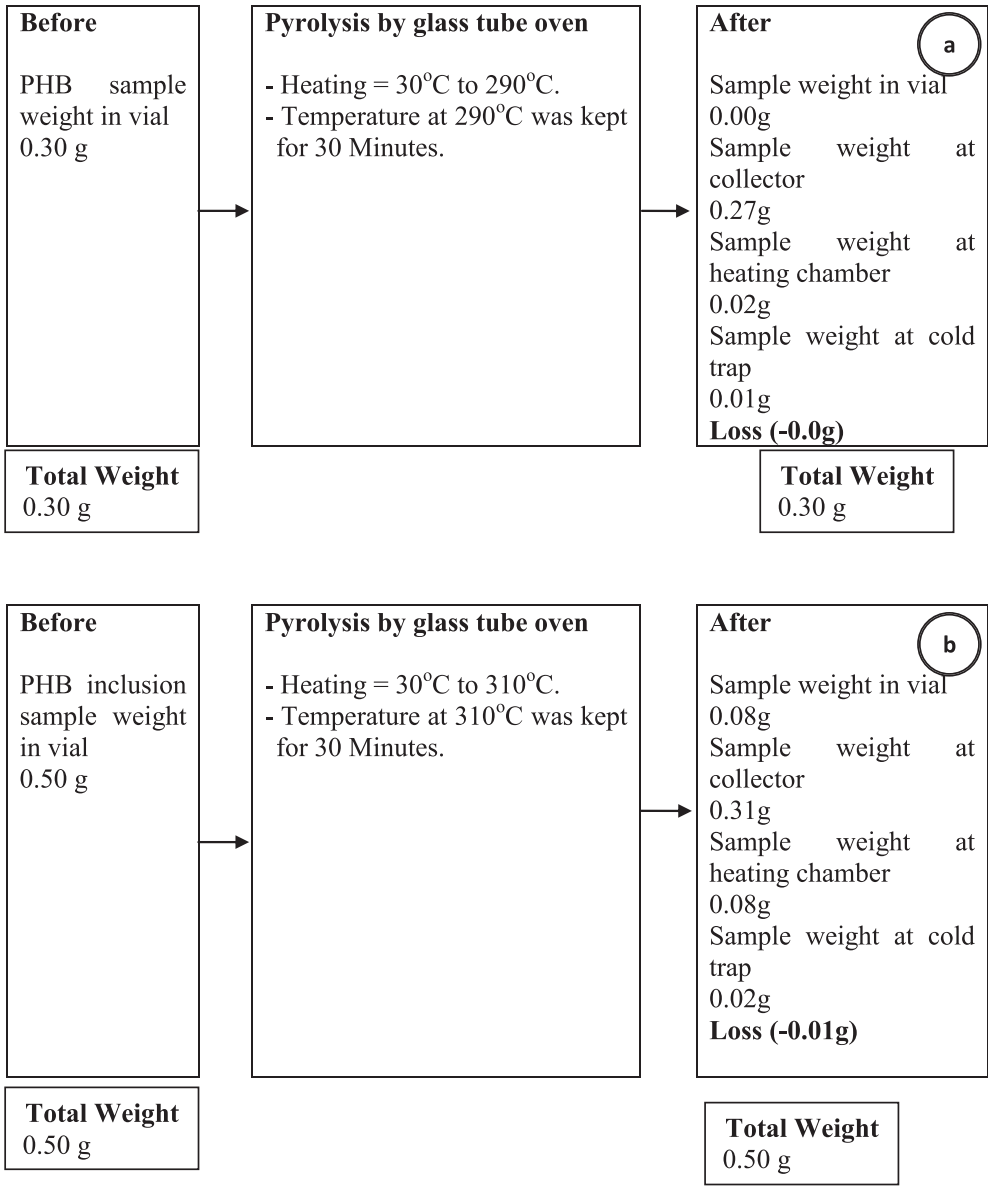

Fig. 5. Mass balance of the (a) PHB, (b) PHB inclusion.

Table 3
Comparison between current production steps of crotonic acid and proposed alternative production of crotonic acid. The route for crotonic acid production from petroleum source is adapted from Arpe (2010).

Industrial petrochemical-based method		Proposed bio-based method	
Steps	Consumables	Steps	Consumables
1) Steam cracking (To convert heavy hydrocarbon, *i.e.* naphtha into ethylene)	a) Saturated hydrocarbon b) Ethylene c) Caustic solution to remove CO_2 and H_2S	1) Fermentation (To obtain bacterial biomass which contain PHB inclusions)	a) Fermentation media as in Section 2.1.
2) Oxidation (To convert ethylene to acetaldehyde)	a) Catalysts: $PdCl_2$ and $CuCl_2$ b) Byproduct: N_2, acetic acid, chlorine-containing compounds.	2) Pretreatment (To separate biomass from fermentation broth and to dry the biomass)	a) No chemical was used. b) Fermentation broth will be treated for wastewater treatment
3) Aldolization (To convert acetaldehyde to acetaldol)	a) Catalyst: dilute caustic soda b) Byproduct: crotonaldehyde	3) Pyrolysis (To obtain crotonic acid)	a) No chemical was used. b) Black residue can be used as biochar.
4) Dehydration (To convert acetaldol to crotonaldehyde)	a) Catalyst: acetic acid.		
5) Oxidation (To convert crotonaldehyde to crotonic acid)	a) Catalyst: copper, thalium, manganese or cobalt salts		

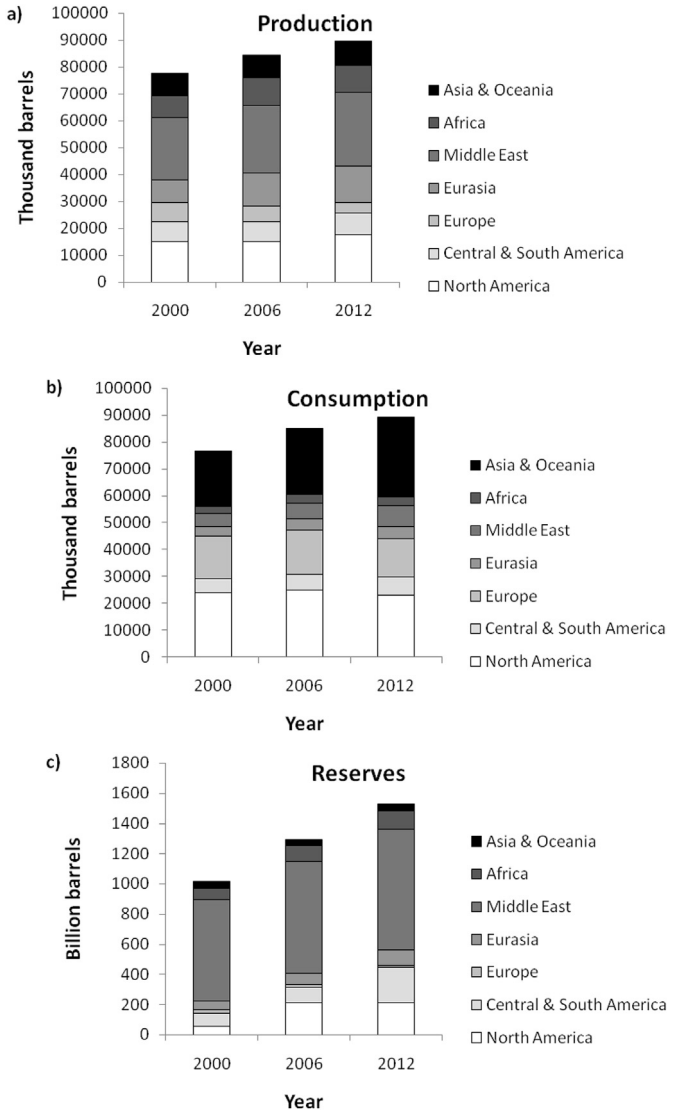

Fig. 6. Estimated global petroleum production (a), consumption (b), and reserves (c). Source: EIA., 2013.

and multiple waste materials discharged from agricultural and food processing industries (Chee et al., 2010a) as depicted in Table 2. Utilization of agricultural biomass such as oil palm frond could reduce the dependence on food crops (Zahari, 2013b). According to Goh et al. (2010), OPF biomass produced in Malaysia in 2007 was approximately 46.8 million tonnes per annum. Therefore, based on calculation by Zahari (2013), this biomass can be processed to produce approximately 5.27 million tonnes fermentable sugars and subsequently converted into 1.56 million tonnes PHB annually. The use of these waste materials is also economically attractive since it does not only serve as carbon source for the fermentation, but also save the cost for waste disposal.

3.3.2. Process steps

According to Table 3, there are five chemical processes required in order to produce crotonic acid from hydrocarbon. Ethylene, which is the primary chemical feedstock, can be obtained from two methods: thermal or steam cracking of hydrocarbon or naphtha. In thermal and steam cracking methods, the hydrocarbon or naphtha is heated in at high temperature, usually 400–500 °C and 1050 °C, respectively. Then, ethylene undergoes partial oxidation process or also known as Hoechst-Wacker process to generate acetaldehyde. Commonly, palladium chloride ($PdCl_2$) and copper chloride ($CuCl_2$) are used in the process to increase the yield of acetaldehyde. Next, acetaldehyde is converted in to acetaldol by aldolization process in tubular flow reactor at 20–25 °C for several hours with caustic soda served as a catalyst and generates crotonaldehyde as the only byproduct. Then, via dehydration in the presence of acetic acid, acetaldol is converted to crotonaldehyde and purification reaction by distillation results in crotonaldehyde with 95% purity. Finally, crotonic acid is produced through oxidation of crotonaldehyde at low pressure (1–5 bar) and temperature (20–45 °C) because reaction at high temperature leads to decomposition of the crotonaldehyde. The use of catalysts such as copper, thalium, manganese or cobalt salts enables the reaction to be conducted at even 20 °C with appeasing yield. Besides, catalysts also can prevent the accumulation of unwanted peroxocompounds (Arpe, 2010). Common yields of this reaction is usually consists of 30% crotonic acid, 1–3% of formic and acetic acids, water and very little amount of isocrotonic acid (Schulz et al., 2003).

In comparison to the petrochemical-based production of crotonic acid, proposed bio-based production of crotonic acid (Table 3) is easier and involves less process steps. Fermentation of PHB biomass has been studied thoroughly and there are various bacteria strains and renewable carbon resources available to be chosen for fermentation (Chee et al., 2010a). Second step of the proposed method requires the biomass to be dried before the pyrolysis can be commenced. The PHB biomass can be dried either by freeze drying or spray drying; the latter is preferred. Even though there are other options such as drying by heating or directly proceeds to pyrolysis after separation of PHB biomass from fermentation media, the feasibility of the process is yet to be examined.

In term of the effect of both methods on the environment, the proposed method is believed to have less contribution to the environmental pollution. This is because the proposed method uses less hazardous chemicals in its process compared to petrochemical-based process. Besides, the proposed method only generates wastewater from fermentation process and the remaining carbon residue after the pyrolysis. The wastewater can be easily treated while carbon residue can be used as biochar which can be used for soil amendment, carbon sequestration, bio-fuel, and wastewater treatment (Roberts et al., 2010). Therefore, the proposed method could be a better option for future crotonic acid production.

3.3.3. Crotonic acid yield

Another aspect to be considered when comparing the current and proposed methods is the yield of crotonic acid produced. As mentioned previously, the production of crotonic acid via

Table 4
Comparison between petrochemical-based and bio-based production of crotonic acid.

Method	Resource	No. of steps	Crotonic acid yield (%)	Estimated selling price (USD)[a]
Petrochemical	Nonrenewable	5	30[b]	6.57–13.13
Bio-based	Renewable	3	63	7.80–11.05[c]

[a] Cost to produce 1 kg of crotonic acid.
[b] Crotonic acid yield from oxidation of crotonaldehyde (Schulz et al., 2003).
[c] Selling price is calculated based on 15–40% gross profit margin (Peters et al., 2003).

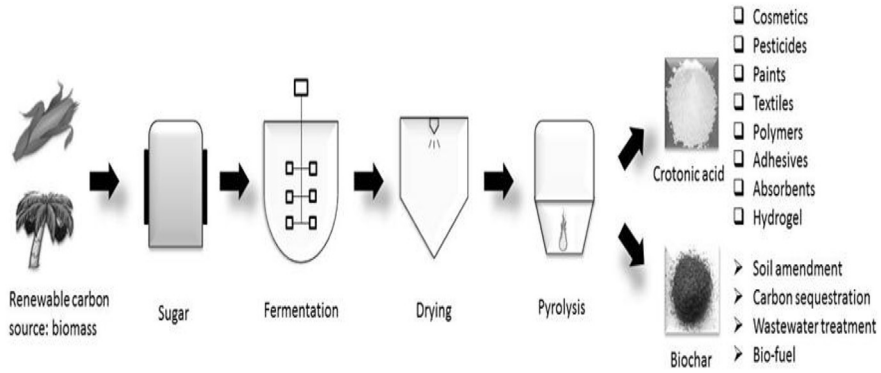

Fig. 7. Schematic diagram of bio-based crotonic acid production via pyrolysis of PHB biomass.

conventional method (oxidation of crotonaldehyde) yield approximately 30% of *trans*-crotonic acid, water, traces of *cis*-crotonic acid and 1–3% of acetic and formic acid (Schulz et al., 2003). Pure *trans*-crotonic acid or *cis*-crotonic acid can be obtained by several methods such as fractional distillation, water crystallization and melt crystallization (Rittner et al., 1990). In contrast to conventional method, pyrolysis of PHB biomass could produce approximately 63% of crotonic acid (Table 4), much higher than the conventional method which may ease purification procedure. In addition, there are plenty of spaces for improvement in term of crotonic acid yield. For instance, Ariffin et al. (2010) reported that the use of magnesium salt as catalyst was able to reduce the activation energy of degradation process and consequently reduced the formation of dimer and trimer. Moreover, the use of catalyst was also able to selectively convert isocrotonic acid and oligomers into crotonic acid and this contributed to higher crotonic acid yield (Ariffin et al., 2010). Aside from that, it is believed that manipulation of pyrolysis parameter will enhance crotonic acid production which is currently in progress. The result obtained will be published in the near future.

3.3.4. Estimation of selling price and market potential of bio-based crotonic acid

In this estimation study, the PHB production process consists of several steps starting with fermentation step, by growing PHB-producing bacteria in nutrient-rich media until high cell density is achieved. After that, fermentation is shifted toward accumulating PHB in bacterial cell by limiting particular nutrients such as nitrogen, phosphorus and oxygen with excess of renewable carbon supply. The fermentation is stopped after 45–50 h with final cell dry weight and PHB content of 125–150 kg/m^3 and 65–70% respectively. Then, separation and extraction are performed (Nonato et al., 2001). As for pyrolysis step, PHB biomass undergoes drying process before being pyrolyzed the required temperature. Finally, the pyrolyzate which contains the desired crotonic acid is collected and recovered (Fig. 7).

As shown in Table 5, total production cost of crotonic acid through pyrolysis of PHB biomass is estimated at USD 6.63 kg^{-1}, and by assuming general range for gross profit margin of 15–40%, sale price range of crotonic acid is USD 7.80–11.05 (Table 4), which is comparable with rough estimation of current petrochemical crotonic acid selling price which is about USD 6.75–13.50. Even though the value obtained is a rough estimation, it still showing a great potential of the proposed bio-based method. In fact, the calculation was overestimated since PHB production cost also covers the cost for recovery. Moreover, the production cost does not consider the revenue that could be obtained from pyrolysis residue which can be used as biochar.

Market for bio-based products is seen to grow year by year. The transition from petroleum-based to bio-based products or economy is driven by several reasons; 1) limited availability and elevated price of fossil resources, 2) pollution caused by petroleum-based industry, 3) abundance of biomass, 4) renewable and sustainable economy (Jong et al., 2012). Biorefinery concept where an integrated system to maximize the conversion of renewable resources especially agricultural biomass into bio-energy, bio-materials and bio-chemicals seems attractive and better option to be an alternative and eventually replace the conventional petroleum-based industries (Jong et al., 2012; Zahari, 2013b). Development of biorefinery systems is also believed to stimulate growth of rural area (Jong et al., 2012) and consequently providing more job opportunities (Zahari, 2013).

Recently, Somleva and colleagues (2013) reported on the potential of crotonic acid as precursor for several important chemicals such as maleic anhydride, acrylic acid, butanol and propylene by appropriate chemical processes such as hydrogenization, oxidation and metathesis. These chemicals and their derivatives have been known to have a wide range of applications and present good market opportunity (Table 6). Currently, they are industrially produced from non-renewable petroleum resource; therefore alternative production of these chemicals from bio-based crotonic acid is potentially useful towards the production of sustainable chemicals. It is anticipated that the production of bio-based products could contribute approximately USD 10-15 billion of revenue for the chemical industries worldwide (Jong et al., 2012). Overall, proposed bio-based production of crotonic acid may be able to reduce dependability to fossil resource thus allowing it to be used in more important sector such as for energy production as well as contributing to more sustainable industry.

Table 5
Summary of the production cost of crotonic acid from pyrolysis of PHB biomass.

Item	Cost (USD)	% of total cost
Raw material cost (PHB biomass)[a]	33,100,000[c]	79.27
Pyrolysis cost (capital & operation)[a]	305,256.20[d]	0.73
General expenses[a,b]	8,351,314.05	20
Total product cost	41,756,570.25	100
Crotonic acid production cost (USD/kg)	6.63	

[a] Cost per 10,000 metric tonne of PHB biomass.
[b] general expenses include administrative cost, distribution and marketing cost, and research and development cost which are estimated to account for 15–25% of total product cost (Peters et al., 2003).
[c] Based on Zahari (2013b).
[d] Based on Bridgwater et al. (2002) and it is assumed based on 20 years of operation.

Table 6
Platform chemicals which can be produced from crotonic acid and their market opportunities (Jong et al., 2012).

Biobased platform chemical	Chemical process from crotonic acid	Market opportunity	Market demand
Maleic anhydride	Oxidation	Maleic anhydride is a precursor for succinic acid which can be converted to various chemical such as 1,4-butanediol (BDO), polybutylene succinate (PBS) tetrahydrofuran (THF).	BDO has market size of approximately 1 million tonnes worldwide.
Butanol	Hydrogenation	Butanol can be used as biofuel, solvent and polymers industries. .	In 2006, it was estimated that global market size for butanol is 2.8 million tonnes.
Acrylic acid	Methathesis	Acrylic acid and derivatives have been used in polymers, adhesives, and resin industries.	Global market size for acrylic acid in 2011 was nearly USD 8 billion and growing 3 to 4 percent every year.
Propylene	Methathesis	Propylene is also an important platform chemical for production of polypropylene and various chemicals.	It has market demand of approximately 50 million tonnes worldwide.

4. Conclusions

It can be concluded that bio-based crotonic acid can be obtained by manipulating degradation behavior of PHB through pyrolysis of bacterial PHB inclusions derived from fermentation using renewable resources i.e. oil palm frond juice as starting raw material (Fig. 7). The proposed method was able to yield 63% of crotonic acid; 30% higher than the conventional petrochemical route by crotonaldehyde oxidation which will consequently simplify the purification process. Besides, the proposed method also offers other benefits such as renewable, simple process and utilization of biomass as raw material could reduce dependency on food crop and decrease operational cost by reducing cost for biomass disposal. Furthermore, the use of PHB as starting material for crotonic acid production provides new route to the application of PHB, as PHB is currently not widely used due to its brittleness and thermal instability. Based on amount of OPF biomass produced in Malaysia and finding of this study, about 1.56 million tonnes of PHB can be produced which can be subsequently converted into 982 ktonnes of crotonic acid by pyrolysis annually. Overall, this study may contribute to better utilization of biomass to be used as renewable source and minimal utilization or release of hazardous materials to the environment through green technology.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jclepro.2014.07.064.

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